CHAPTER 1

General Introduction
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INTRODUCTION

Chronic kidney disease (CKD) is expected to be the 5th most common non-communicable disease in 2050 globally. It is recognized as a major health-care problem due to its association with cardiovascular mortality, costs to the healthcare system, and patient well-being. An estimated 1.23 million people die annually due to CKD, a 33% increase over the past decade. Treatment of advanced CKD, particularly end-stage kidney disease (ESKD), is possible with renal replacement therapies (RRT) including dialysis techniques and transplantation, which are also chronic therapies with significant side-effects to patients, and extremely costly to health systems.

Inflammation remains a major mechanism contributing to the progression of the various kidney diseases towards ESKD. It is also involved in the high burden of morbidity and mortality in patients with advanced CKD, contributing to both increased infectious and cardiovascular (CV) risk, and a premature aged phenotype. The prototype of inflammatory kidney diseases are the glomerulonephritides, which represent the third most common cause for ESKD in the United States, and where disorders of the adaptive immunity triggered by the activation of the innate immunity lead to the production of an inflammatory microenvironment. Particularly, the influx of inflammatory cells such as neutrophils, macrophages, dendritic cells, T-cells, B-cells and mastocytes, results in progressive damage and disruption of the glomerular architecture, leakage of both erythrocytes and proteins to the urinary space, and eventually fibrosis. Among this group of kidney diseases, IgA nephropathy (IgAN) is the most common and comprises a wide spectrum of clinical manifestations, ranging from asymptomatic patients with only minor urinary abnormalities (including microscopic hematuria and/or low-degree proteinuria), to those with rapidly progressive deterioration of renal function or nephrotic proteinuria. Approximately 30% of IgAN patients develop ESKD within 20 years. Its relevance is further underlined as it is the most common cause of ESKD in young adults. Concerning the pathogenesis, a multi-hit theory with four steps has been proposed for IgAN, starting with defective glycosylation of IgA1 that results in overproduction of galactose deficient IgA1 (Gd-IgA1), the production/development of IgG or IgA1 anti-Gd-IgA1 autoantibodies, and mesangial deposition of immune complexes formed in circulation or in situ. This deposition can be nephritogenic; leading to increased mesangial cell proliferation, activation of the complement system and various inflammatory responses.
In advanced CKD, inflammation has a multifactorial etiology with interactions with a number of factors that emerge in the uraemic milieu, including exogenous factors (i.e. the dialysis membrane and the central venous catheter); cellular factors (i.e. oxidative stress and cellular senescence); tissue factors (i.e. hypoxia, fluid and sodium overload); microbial factors (i.e. immune dysfunction and gut dysbiosis); and finally, retention of uraemic toxins (i.e. indoxyl sulfate and p-cresol, calciprotein particles and advanced glycation end products). Although considerable progress has been made in survival rates of dialysis patients, significant cardiovascular morbidity and mortality are still faced by this patient population. In the US, the mortality approaches 40% in the first year of dialysis for patients over 75 years-old, and even those with a better prognosis suffer from declining functional status and are faced with multiple hospital admissions. Furthermore, although outcomes in other western countries are generally better than in the US, dialysis mortality after two years is still around 20% in European cohorts. Traditional risk factors such as hypertension, dyslipidemia, and diabetes can only partly explain the poor prognosis of dialysis patients. Other, non-traditional, risk factors such as oxidative stress, endothelial dysfunction and chronic inflammation, are therefore important potential contributors to the high cardiovascular risk in this population.

In order to improve the high morbidity and mortality rates in dialysis patients, the chronic inflammation status present in these patients, which has been associated with a state of acquired immune dysfunction, metabolic and nutritional derangements, depression and poor cognitive status, and increased infectious and cardiovascular risk, must be tackled. Experimental and clinical studies have suggested for a long time that targeting inflammation, without affecting lipid levels, will reduce cardiovascular risk. Yet, until recently, this inflammatory hypothesis of atherothrombosis was unproved. Ridker et al. demonstrated for the very first time in a randomized, double-blind trial involving over 10,000 patients with a history of cardiovascular disease, that anti-inflammatory therapy reduces recurrent cardiovascular events. This landmark study has opened the door for other patient populations and different anti-inflammatory drugs to be used to reduce cardiovascular risk.

The complement system is a major contributor to the chronic inflammation occurring both at a local level in glomerulonephritides, and systemically in the uremic state that characterizes the advanced CKD stages. As a major part of the innate immune system, the complement system consists of a cascade of circulating proteins as well as cell surface proteins. Complement proteins circulate as zymogens which are activated...
after cleavage. The early activated proteins form proteolytic enzymes that cleave downstream proteins, and the amplification of this cleavage process can generate large numbers of downstream fragments. Due to the potent effects that these activation products may have, this process needs to be controlled by a family of regulatory proteins, or complement inhibitors. The complement system was originally perceived as a defense system against pathogens. However, this traditional view has evolved over the past decades. Additional implications for the complement system have been described in different disease processes as well as its importance in homeostasis. This homeostasis is related to the crucial role taken by complement in the removal of the apoptotic/necrotic cellular debris and balancing inflammation. Various complement components, such as C1q, mannose-binding lectin (MBL), ficolins, and properdin, act as important opsonins ensuring recognition and removal of cellular material by phagocytes. Equally important for this removal process is the inhibition of excessive complement activation on altered self, through the binding of complement inhibitors.

Complement activation can be initiated via three pathways. The classical pathway (CP) activation occurs when antibody-antigen complexes bind to C1q, the first protein of the cascade, leading to activation of C1r and C1s. This C1q,r,s complex cleaves C4 and C2, generating C4bC2a also known as the C3-convertase. The lectin pathway (LP) is activated when MBL, ficolins or collectins bind to specific carbohydrate patterns uncommon in the host, and initiate the enzymatic activity of MBL-associated serine protease (MASP). The MASPs, particularly MASP-2, cleave C4 and C2, also generating C4bC2a. The alternative pathway (AP) is continuously activated in small amounts leading to the spontaneous hydrolysis of C3, C3(H2O), thereby exposing the thioester bond, which is highly reactive and has C3-convertase activity. C3b generated by the different C3-convertases then combines with Factor B. Next, Factor D cleaves Factor B to form C3bBb, which further cleaves additional C3. If C3b generated by this convertase binds to a nearby surface, such as bacteria, yeast cells or damaged host tissues, which is not capable of inactivating it, it can, itself, form more C3bBb, leading to further amplification of the alternative pathway. Each of the C3-convertases, either C3bBb or C4bC2a, can combine with an additional C3b to form the C5-convertase. C5-convertases catalyze the cleavage of C5 into C5a and C5b. The latter interacts with C6, C7, C8, and finally C9 leading to the formation of C5b-9 (the terminal complement complex or membrane attack complex), a structure that creates functional pores in membranes leading to cell lysis.
The kidney has a close link with the complement system and for unknown reasons is a highly susceptible organ to complement-induced damage. Within Nephrology, there are a wide spectrum of complement-mediated diseases and the complement system can be the direct cause or the aggravating factor in the pathogenesis of renal diseases. Tissue injury from complement activation may occur during an appropriate response to pathogens, but may become significant if the response is overwhelming. Therefore, the same downstream complement fragments that help to eliminate pathogens during infections also cause tissue injury in autoimmune and inflammatory diseases.

In patients with an impaired ability to regulate complement, a disease flare can be triggered by physiologic complement activation. Mild insults such as transient infections may lead to chronic complement-mediated disease. IgAN may be considered an example of chronic AP dysregulation. IgG or IgA1 autoantibodies bound to galactose-deficient IgA1 form immune complexes, deposit in the mesangium and cause disease. In this process, minor differences in the ability to regulate the AP probably affect the rate of disease progression over time. In fact, genetic variations and differences in expression levels of factor H and the Factor H-related proteins (FHRs) have been demonstrated to influence the disease. However, the LP has also been implicated in IgAN since tissue deposition of C4d, an activation fragment of C4 and established marker of LP activation, has been identified in a subgroup of patients with worse prognosis.

The complement system has also been shown to contribute to the pro-inflammatory uremic milieu and cardiovascular risk in patients with ESKD. Complement’s involvement in dialysis has been traditionally associated in the past to the more commonly reported reactions to non-biocompatible hemodialysis (HD) membranes. This would occur in the early phases of the dialysis sessions, where high levels of C3a and C5a would be found in relation to acute neutropenia. C3a and C5a are small split fragments of complement activation known as anaphylatoxins, and particularly C5a is an extremely potent inflammatory mediator. These membrane reactions were shown not only to provoke acute and transient effects but also lead to chronic complement activation and inflammation. With the advent of the “biocompatible” dialysis era, interest in the involvement of the complement system in HD has waned. However, in recent years, increasing awareness of the importance of chronic inflammation caused by the procedure itself and its commonly associated drug therapies, such as intravenous iron, and the reappearance of various reports on dialyzer reactions even with the use of the so-called biocompatible membranes, has promoted the resurgence of the
interest on the involvement of the complement system in HD.\textsuperscript{6,39–41} Evidence for Ficolin-2 adsorption by polysulfone membranes and its association with inflammation in HD,\textsuperscript{42} the predictive value of MBL for cardiovascular (CV) outcomes in HD patients,\textsuperscript{33} as well as the association of high levels of C3 and extreme levels of sC5b-9 with subsequent CV events in a cohort of 260 patients on HD,\textsuperscript{43} has confirmed that the study of complement in HD is an exciting and expanding field in Nephrology. In peritoneal dialysis (PD), the available evidence on the involvement of complement remains several steps behind, although early studies repeatedly reported findings that suggest local and, even perhaps, systemic complement activation in PD patients.\textsuperscript{44–46} Biocompatibility of the dialysis solutions is a matter of intense research in PD in the last years,\textsuperscript{47,48} and the preservation of both the peritoneal membrane and renal residual function is vital for the success of the technique and consequently to patient outcomes. However, evidence for the potential clinical relevance of complement to influence these outcomes is still lacking, and further studies are still needed to improve the understanding of the complement system pathophysiology in PD.

Research into the complement system has seen a revival in the last 20 years in nephrology. The spectacular effects of the blockage of the terminal component C5 with the monoclonal antibody eculizumab in the once untreatable atypical hemolytic-uremic syndrome (aHUS) has encouraged more research on the complement system in various kidney diseases. Ongoing trials are evaluating the use of complement inhibitors in various diseases beyond those directly related to complement dysregulation, such as aHUS and C3 glomerulopathy, including ANCA-associated vasculitis, IgAN, immune-complex membranoproliferative glomerulonephritis, lupus nephritis, membranous nephropathy and allograft antibody-mediated rejection.\textsuperscript{49} Eventually, targeting the complement system will also happen in maintenance dialysis patients.
Scope of the thesis

The aim of this thesis was to contribute to the growing body of knowledge on the role of the complement system in the common inflammatory kidney disease IgAN, and in advanced CKD patients on HD or PD maintenance therapies.

Chapter 2 focuses on the role of glomerular C4d as a biomarker of disease progression in IgAN, and compares it with a range of previously studied biomarkers of worse prognosis, including (i) transglutaminase-2 (involved in kidney pro-fibrotic processes); (ii) p-ERK1,2 (previously shown to be involved in inflammatory processes in IgAN); and (iii) CD3 (T cell marker). In Chapter 3, building on the growing importance of the previously neglected vascular compartment in IgAN biopsy classification systems, C4d was evaluated in arterioles of 126 IgAN patients and its association with other established markers in IgAN and outcomes of this disease was studied. In Chapter 4, an overview of the body of evidence on the role of the complement system in both HD and PD was reviewed. In Chapter 5, intradialytic complement activation was assessed in a cohort of HD patients and associated with cardiovascular outcomes. Furthermore, to explore the causal relationship between complement activation and subsequent inflammation and coagulation, we developed an ex-vivo model of HD and tested the effect of complement inhibition on inflammation and coagulation. Considering that iron administration is one of the most commonly and repeatedly administered drugs among the hemodialysis patients, Chapter 6 investigates the possible effects of intravenous iron formulations on complement activation. In Chapter 7 the role of a soluble form of complement regulator CD59 was studied in a cohort of PD patients, particularly the associations between systemic and peritoneal levels, and its associations with patient baseline characteristics and outcomes. Finally, in Chapter 8, we performed a comprehensive investigation of the complement system in PD, including the presence of both components and activation products at a systemic and local level. We compared this complement profile with the one found in HD, non-dialysis CKD and healthy controls, and investigated the pathways responsible for complement activation in PD. Furthermore, possible associations of complement activation with baseline clinical characteristics and clinical outcomes were investigated in our cohort.
REFERENCES


PART A

The Complement System in IgA Nephropathy